ORIGINAL PAPER

Bioactive Compounds Obtained from Oilseed By-Products with Subcritical Fluids: Effects on *Fusarium verticillioides* Growth

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Abstract

Recovery of bioactive compounds from wastes is gaining interest because they could add value to by-products arising from, for example, the oil extraction processes. In this work, green solvent extraction (water/ethanol under sub-critical conditions) was used to obtain bioactive compounds from peanut, sesame and pistachio agro-industrial by-products. Extracts were analyzed in their overall chemical composition and tested on growth, ergosterol and fumonisin FB_1 production by *Fusarium verticillioides.* The effects of the extracts on fungal growth rate and biochemical markers were not univocal, and could be associated to differences in their chemical profiles. Extracts obtained from peanut skin—composed mainly by monomeric and dimeric flavonoids—caused significant reductions in fungal growth rate but increased FB_1 production. Extracts from sesame seeds—dominated by furofuran-type lignans—did not have a clear inhibitory effect on growth rate but strongly reduced both FB_1 and ergosterol production. Extracts from pistachio nuts—characterized by monomeric flavonoids and gallic acid derivatives—showed minor effects on both fungal growth rate and biochemical markers. Sub-critical fluid extraction of peanut skin and defatted sesame seeds may provide an efficient method to obtain extracts rich in phenolic and lignan compounds with potential use as antifungal agents.

Graphic Abstract
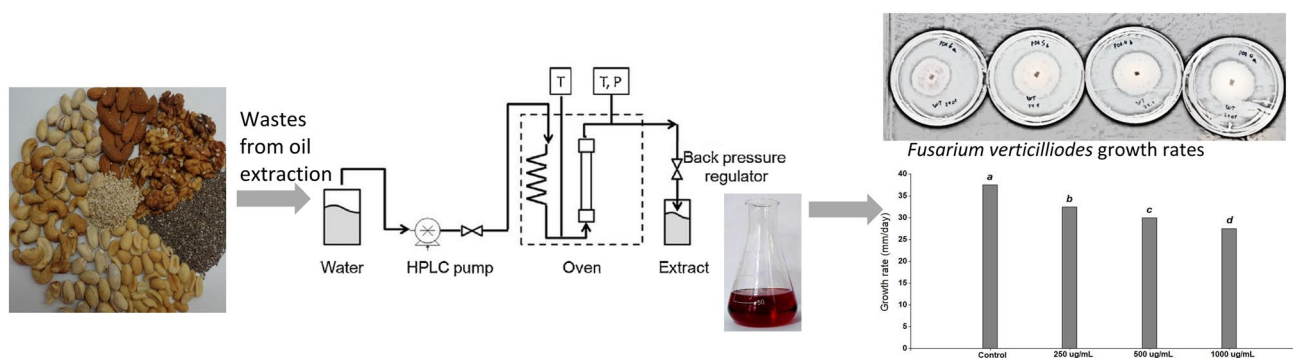
Fusarium verticilliodes growth rates

Abbreviations

FB_1	Fumonisin B_1
GA	Galic acid

HPLC–ESI–MS/MS	High pressure liquid chromatography–electrospray ionization–mass spectroscopy
PNC	Pistachio nuts cake
PS	Peanut skin
SSC	Sesame seed cake
SFE	Sub-critical fluid extraction
TPC	Total phenol content

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Statement of Novelty

Currently the agricultural produces large quantities of plant wastes that contain bioactive molecules that can be assessed as functional ingredients, so this is a sustainable alternative to the byproducts exploitation. In particular 'cakes' are obtained after oil removal in the oil food industry and these are a source of bioactive compounds. Fungal contamination is an important crops constraint, so synthetic fungicides are widely used. An alternative is the use of botanical products, which are considered of minimum risk. In the current context, the search for sustainable, non-polluting extraction technologies for making natural biopesticides add even more value to the recovery of wastes biocompounds. In this work, sub-critical fluid extraction of peanut skin and defatted sesame seeds may provide an efficient method to obtain extracts with potential use as antifungal agents.

Introduction

Currently agricultural and food industry produces billions of tons of plant-derived wastes that come from the cultivation, harvest, post-harvest and others food processing. It's residues contain a wide and diverse range of bioactive molecules such as: proteins, fibres, polysaccharides, phenolic and flavour compounds among others. These differents phytochemicals can be valorized as functional ingredients in food, pharmaceutical and a vast array of products [1–3]. The use of these recovered bioactive compounds shows a sustainable alternative to the vegetable byproducts exploitation as an economic source and, at the same time, contributing to the efficient waste management.

In particular cakes or meals are obtained after oil removal by cold pressing of different seeds and nuts used in the edible oil industry. Their chemical composition varies due to the differences in crop, in harvest time and in the oil extraction methods [4]. Recovery of bioactive compounds from these materials is gaining interest because they could add value to by-products arising from the oil extraction processes [3]. Previous works studied oilcakes mainly as source of food nutritional supplements [4] and antioxidant compounds [5, 6]. But has also been shown that they could be used in enzymes, antibiotics, biopesticides and vitamins production [4, 7].

On the other hand, fungal contamination is one of the most important constraints to production and storage of many crops. Various *Fusarium* spp. have been reported to be seed borne in corn, sorghum and soybean [8]. *Fusarium verticillioides* is one of the main pathogen fungi of corn and produces a group of micotoxins namely fumonisins among which the FB_1 is believed to be the most toxic. For these reasons, International legislations limit the presence of these toxins in human food and animal feed [9].

In this context synthetic fungicides such as benzimidazols are widely used to control *Fusarium* spp. These compounds, however, have some drawbacks including the development of pathogen resistance [10] and the possible toxicity for animals [11]. An alternative to control pests of stored grains as is the use of natural products derived from botanic materials, which are considered to be of minimum risk [12]. So over recent years several studies have searched new antifungal materials from natural sources and many antimicrobial compounds coming from plants have been identified. In this context some authors have reported the antifungal activity of essential oils and phenolic components against filamentous fungi [13, 14] and in particular on *Fusarium verticillioides* [15–17].

Many oil-bearing seeds and nuts, such as sesame (*Sesamum indicum* L.), peanut (*Arachis hypogaea* L.) and pistachio (*Pistacia vera* L.) are known to produce a vast array of bioactive compounds. In particular lignans are found at high quantities in sesame cake [18, 19] and their antimicrobial activity has been reported for many fungal species including *Fusarium graminearum* [20, 21], *F. moniliforme, F. sporotrichum, Aspergillus flavus, A. niger* [22], *Alternaria alternate* and *A. citri* [23]. In the case of peanut skin—a by-product of the industrial blanching operations—is found to be an unusual source of several phenolic and polyphenolic substances [24] having strong antioxidant, antimicrobial and antifungal properties [25–27]. Finally Rajaei et al. [28] have reported antimicrobial properties of pistachio phenolics against Gram positive and Gram negative bacteria and fungi (*Candida albicans* and *Neurospora intermedia*).

Traditionally, extraction of phenolics or other bio-active compounds from different vegetable matrices has been carried out by means of conventional solid–liquid extraction methods (maceration, Soxhlet, etc.) with different organic solvents. Their use, however, may have some limitations including long extraction times and large amounts of solvents that often are prohibited by food regulations. Recent investigations have been focused on green solvent extraction using water and ethanol at high pressure and temperature conditions. The feasibility of the so called sub-critical fluid extraction (SFE) for obtaining different kinds of natural compounds is widely documented [29] and is a technology consistent whit the sustainability objective.

The present study was aimed to evaluate the effects of sub-critical fluid extracts obtained from sesame, peanut and pistachio by-products on growth, ergosterol and fumonisin FB_1 production by *Fusarium verticillioides*.

Materials and Methods

Plant Material

Peanut skin (PS) was obtained from Runner-type peanuts by means of a typical industrial blanching process (90 °C, 10 min). Sesame seeds (SS) and pistachio nuts (PN) were obtained from commercial plantations located at NW Argentina. These two latter materials were partially defatted by screw-pressing (Komet, Model CA 59 G, Mönchengladbach, Germany) according to procedures described elsewhere [30]. The corresponding defatted cakes (SSC and PNC) were milled (cutting mill, Moulinex, France) and sieved (automatic screen EJR 2000 Zonytest, Argentina) to obtain uniform particle size (mean value 0.5 mm) and then stored in amber glass containers at − 20 °C under nitrogen atmosphere.

Sub-critical Fluid Extraction

Defatted materials (PS, SSC and PNC) were submitted to SFE using the experimental setup reported elsewhere [18, 25]. The solvents employed were distilled water and absolute ethanol. The extraction conditions (temperature, mass flow, pressure and ethanol content) were fixed according to previously reported results [18, 25]. For each material, the extract obtained was collected and centrifuged at 10,000 rpm for 20 min. The solvent was removed using a rotary vacuum evaporator at 40°C and the residue was dried by lyophilization. Dry extracts (DE) were stored in amber glass bottles under nitrogen at − 20°C until use.

Extracts Analyses

Total phenol content (TPC) of PS, SSC and PNC extracts was determined using the Folin–Ciocalteau reagent according to Singleton et al. [31]. TPC was quantified with a standard curve using gallic acid (GA) and it was expressed as mg GA/kg DE (in supplementary date).

The composition of the extracts was analyzed by HPLC–ESI–MS/MS using an Agilent 1200 Series system (Agilent Technologies, Santa Clara, CA, USA) under analytical conditions used previously [25]. Tentative identification of lignans and phenolics was based on their retention times (Rt), elution order, UV–Vis spectra and MS fragmentation spectra as compared with commercial standards, in addition to those reported in the literature [24, 31, 32]. The Compass version 3.1 software and Data Analysis version 4.1 software were used for data acquisition and processing respectively.

Fungal Strain

An isolate of *F. verticillioides* M3125 (provided by Dr. Robert Proctor, United States Department of Agriculture, was used for all experiment. This fumonisin producing strain was isolated from maize in California [33].

Antifungal Properties

The antifungal activity of the extracts was determined by the radial growth of the fungal colony following a methodology proposed by Pizzolitto et al. [26]. Briefly, inoculum was prepared by growing on PDA (Potato dextrose agar) medium for 7 days at 25 °C to allow profuse sporulating. The conidial concentration (10^6 conidia/mL) was standardized using a hemocytometer. The PS, SSC and PNC extracts were tested at different concentrations of 250, 500 and 1000 μg/mL. The dry extracts (DE) were first dissolved in 100 μL of dimethylsulfoxide (DMSO) and then added to the autoclaved based medium. In control treatments, the equivalent amount of DMSO was added to the culture medium. PDA plates (90 mm) were inoculated centrally with 10 μL of the spore suspension and were incubated for 7 days at 25 °C. The experiments were repeated thrice. The colony area was determined by periodical measurement. Colony diameters versus time were plotted and radial growth rates (mm/day) were evaluated from the slope by linear regression. The lag phase was calculated as the abscissa from growth rate curves.

Effect on FB1 Production

Maize grains were used as substratum in order to determine Fumonisin B_1 (FB_1) production [26]. Briefly, the grains (25 g) were placed into 100 mL glass vials and sterilized in autoclave for 15 min at 121 °C. Three concentrations (250, 500 and 1000 μg/mL) of extracts were prepared by dissolving DE in DMSO and mixed with water (7.5 mL). Each extract solution (7.5 mL) was added to seed-containing vials, so seeds reached 30% humidity. The 30% humidity was calculated through previous assays taking into account the initial grains moisture (data not shown). A 50 μL aliquot of conidial suspension of *F. verticillioides* (10^6 conidia/mL) was inoculated on maize grains in each individual vial. The vial content was homogenized in a shaker for two minutes. Samples were then incubated 28 days in absence of light at 28 °C, and every 3 days the vials were shaken. Control vials were prepared in similar treatment conditions but without added extracts. The experiments were repeated five times.

Fumonisin B1 Quantification

The quantitative determination of FB_1 was done using the method described by Shephard et al. [34], with some

modifications. After incubation, samples of fermented maize were sterilized in autoclave for 15 min at 121 °C and then dried in vacuum oven at 60 °C until constant weight was achieved. Ten grams of maize were grounded and extracted with ultrapure water by shaking the mixture of powder and water for 2 h. Samples were centrifuged at 9000 rpm and filtered using filter paper. A 500 μL aliquot of this aqueous extract was diluted with 500 μL acetonitrile. Fifty μL of this mixture was derivatized with 200 μL of *o*-phthaldialdehyde. Derivatized samples were analyzed using Perkin-Elemer HPLC equipped with a fluorescence detector following the method described by Pizzolitto et al. [26]. The data analysis of FB_1 was carried out by comparing the peak areas obtained from aqueous extracts with those corresponding to the analytical standards of FB_1 (5.135, 2.567 and 1.283 g/mL).

Ergosterol Quantification

Ergosterol extraction was carried out according to Dambolena et al. [35] and quantification was assayed by HPLC (Perkin-Elemer, Shelton, CT, USA) and UV detection at 282 nm. An analytical reverse-phase column C18 (150 mm × 4.6 mm internal diameter, 5 mm particle size) was used. The mobile phase was acetonitrile/methanol (80:20), at a flow rate of 1.8 mL/min. Ergosterol identification was carried out by comparison of the retention time and standard curve constructed with the pure standard compound (Sigma-Aldrich, St. Louis, MO, USA) and expressed as μg/g DE.

Statistical Analyses

Data were analyzed by analysis of variance (ANOVA). Normality of data was evaluated using the Shapiro–Wilk test. Comparisons between treatments were determined by Duncan test. All analysis were conducted using INFOSTAT/ Professional 2005p.1 program (FCA-UNC, Argentina). The treatments were considered significantly differents at p values < 0.05.

Results and Discussion

Extract Analyses

The average contents of TPC in PS, SSC and PNC dry extracts (DE) were found to be 360, 120 and 140 mg GA/kg DE, respectively. Results from HPLC–ESI–MS/MS analyses are shown in Tables 1, 2 and 3.

The phenolic profile in PS extract (Table 1) was dominated basically by a number of procyanidin dimers isomeric forms consisting entirely of flavan-3-ol monomers, which can be deducted by their [M−H]$^-$ signal at *m/z* 575 and main MS/MS fragments (*m/z* 285 and 449) [36]. Moreover,

three proanthocyanidin dimers were found. However trimers, tetramers and pentamers of both procyanidins and proanthocyanidins [36, 37], were not detected in the present study. In addition, two flavanols (catechin and epicatechin), two flavones (chrysin and luteolin), one flavonol (quercetin) and one *o*-methylated isoflavone (biochanin A) were found at lesser amounts. In accordance with Ma et al. [24], flavonoid glycosides were almost missing; only one glycosilated isoflavone was recognized as a minor component.

Nineteen compounds—mainly lignans and different kinds of phenolic compounds—were identified in SSC extract (Table 2). On the basis of their structural patterns, the former corresponded to furofuran-type lignans which are the predominant lignans in sesame seeds [38]. Four subtypes namely sesaminol, sesamolinol, sesamin and sesamolin were identified based on elution order, accurate mass measurements and MS/MS fragmentation patterns. Sesaminol was found to be glycosylated; this is in agreement with data reporting sesaminol glucosides as the most abundant glycosylated lignans in sesame [38, 39]. The remaining lignans were present as aglycons. They comprised two sesamolinol isomers and three sesamolin isomers showing pseudomolecular [M−H]$^-$ ions at 371 and 369, respectively, in agreement with data reported elsewhere [40]. In addition, three well-known epimeric furofuran lignans (sesamin, episesamin and diasesamin) were also found. The identity of these latter compounds was based on characteristic [M−H]$^-$ signal at *m/z* 353 as reported earlier [38, 40]. Four hydroxycinnamic acid derivatives including two simple phenolic acids (syringic and ferulic acids) and dimers of both caffeic and ferulic acids were found. Syringic and ferulic acids were identified by comparing their retention times and characteristic spectral data with those of authentic standards. Dicaffeic acid and diferulic acid were identified by their typical [M−H]$^-$ signals at *m/z* 341 and 385, and major MS/MS fragments at *m/z* 179 and 297, respectively. Flavonoid compounds and derivatives included the monomeric flavonoids namely apigenin-7-methylether (flavone), epicatechin-3-*O*-galato (flavanol), 4′-hydroxyflavanone and genistein (isoflavone), and one procyanidin dimer which was tentatively identified as B-type dimer on the basis of a typical [M−H]$^-$ signal at *m/z* 577.

Seventeen phenolic compounds—mainly flavonoids and gallic acid derivates—were identified in PNC extract (Table 3). Flavonoids included two isoflavones (daidzein and genistein, this latter as glycosilated derivative), one flavanone (naringenin), and two flavonols (quercetin and kaempferol, this latter as two hexoside isomers). All these flavonoid-type compounds were previously identified in seeds and hulls of pistachio from different origins and cultivars [32]. In agreement with data reported previously [32], three peaks with typical [M−H]$^-$ signals at *m/z* 296, 509 and 403 were found; due to the formation of a major and

Table 1 HPLC-ESI–MS/MS analysis of subcritical fluid extracts obtained from peanut skin

Rt (min)	Compound	Molecular formula	[M−H]⁻ experimental (m/z)	[M−H]⁻ theoretical (m/z)	[MS²[M−H]⁻] (m/z)	Relative abundance
7.7	Quinic acid	$C_7H_{12}O_6$	191.0458	191.0561		++
9.9	Proanthocyanidin dimer (Isomer 1)		573.1573		262–381–531	++
12.2	Proanthocyanidin dimer (Isomer 2)		573.0607		289–453	++
13.2	Catechin	$C_{15}H_{14}O_6$	289.0725	289.0707	203–245	+++
13.3	Proanthocyanidin dimer (Isomer 3)		573.0775		285–411	++
13.5	Procyanidin dimer-Type A (Isomer 1)	$C_{30}H_{24}O_{12}$	575.0784	575.1184	285–411–447	+++
13.6	Epicatechin	$C_{15}H_{14}O_6$	289.0521	289.0707	203–245	+++
13.9	Procyanidin dimer-Type A (Isomer 2)	$C_{30}H_{24}O_{12}$	575.0775	575.1184	285–411	+++
14.5	Procyanidin dimer-Type A (Isomer 3)	$C_{30}H_{24}O_{12}$	575.0831	575.1184	285–449	++++
14.7	Procyanidin dimer-Type A (Isomer 4)	$C_{30}H_{24}O_{12}$	575.0815	575.1184	na*	++++
15.0	Procyanidin dimer-Type A (Isomer 5)	$C_{30}H_{24}O_{12}$	575.0841	575.1184	285–449	++++
15.9	Procyanidin dimer-Type A (Isomer 6)	$C_{30}H_{24}O_{12}$	575.0798	575.1184	na	++++
16.4	Procyanidin dimer-Type A (Isomer 7)	$C_{30}H_{24}O_{12}$	575.0809	575.1184	285–449	++++
16.8	Procyanidin dimer-Type A (Isomer 8)	$C_{30}H_{24}O_{12}$	575.081	575.1184	285–449	++++
17.0	Caffeic acid	$C_9H_8O_4$	179.0473	179.0339		+
17.3	Procyanidin dimer-Type A (Isomer 9)	$C_{30}H_{24}O_{12}$	575.0813	575.1184	285–449	++++
19.4	Procyanidin dimer-Type A (Isomer 10)	$C_{30}H_{24}O_{12}$	575.0766	575.1184	285–449	+++
20.3	Dihydroxicoumarin	$C_9H_6O_4$	177.0106	177.0182	133	++
25.4	Luteolin	$C_{15}H_{10}O_6$	285.0202	285.0394	199–241	++
26.7	Quercetin	$C_{15}H_{10}O_7$	301.0349	301.0343	179	++
29.5	Luteolin methyl ether (Diosmetín)	$C_{16}H_{12}O_6$	299.0541	299.055	284	+
30.4	Chrysin	$C_{15}H_{10}O_4$	253.033	253.0495	209	+
31.4	Genistein	$C_{15}H_{10}O_5$	269.0505	269.0444	na	+
30.8	Isorharmentin (Quercetin methyl ether)	$C_{16}H_{12}O_7$	315.047	315.0499	300	++
35.9	Biochanin A	$C_{16}H_{12}O_5$	283.0608	283.0601	268	++

Relative abundance of the metabolites identified is indicated by + (0–5%), ++ (5–20%), +++ (20–50%) and ++++ (50–100%)

*Not available

characteristic daughter ion at m/z 169, which is attributed to deprotonated gallic acid, they were tentatively identified as gallic acid derivatives. Two peaks showing typical [M−H]⁻ signal at m/z 483 and a major fragment at m/z 331 were also detected; they may be attributed to isomeric forms of digalloyl esters of hexose varying in the points of attachment (linkage) of the galloyl structures to the hexose. On the basis of molecular weight and mass spectra data [M−H]⁻ signal at m/z 577, presence of an intense ion at m/z 289), peaks at 27.9 and 31 Rt were tentatively identified as procyanidin B-type dimers. Caffeoylquinic acid was certainly identified based on the [M−H]⁻ signal at m/z 353 and formation of a characteristic product ion at m/z 191 (deprotonated quinic acid). Only one anacardic acid derivative was detected; based on the [M−H]⁻ signal at m/z 341 and a major product ion at 297 it was identified as (15:3) anacardic acid.

To summarize at this point, the chemical profiles of the extracts obtained by means of the described SFE process were dominated by monomeric and condensed flavonoids, particularly procyanidin and proanthocyanidin oligomers (PS), furofuran-type lignans (SSC), and several monomeric flavonoids and gallic acid derivatives (PNC).

Antifungal Properties

The effects of PS, SSC and PNC extracts on *F. verticillioides* growth rate are shown in Fig. 1. In the presence of PS extract, fungal growth rate was significantly delayed (Fig. 1a). The response was concentration-dependent; extract concentrations of 250, 500 and 1000 µg/mL caused inhibition percentages of approximately 31, 47 and 63%, respectively, in relation to the control treatment. These inhibition percentages are higher than those reported by Pizzolito et al. [26] who found a maximal fungal growth reduction (28%) using a purified PS extract (non-specified composition) at 500 µg/mL.

Looking at the composition of PS extract we tested here, it can be seen that most of the components are monomeric

Table 2 HPLC–ESI–MS/MS analysis of subcritical fluid extracts obtained from sesame seeds cake

Rt (min)	Compound	Molecular formula	[M−H]⁻ experimental (m/z)	[M−H]⁻ theoretical (m/z)	[MS² M−H]⁻ (m/z)	Relative abundance
7.3	Dicaffeic acid	$C_{18}H_{14}O_7$	341.0619	341.0656	179	+
10.0	Apigenin-7-methyl ether	$C_{16}H_{12}O_5$	283.0545	283.0601	221	+
12.2	Sesaminol diglucoside	$C_{32}H_{38}O_{17}$	693.2633	693.2025	325–531	+++
12.4	Sesaminol monoglucoside	$C_{26}H_{28}O_{12}$	531.2043	531.1497	369	++++
14.1	Diferulic acid	$C_{20}H_{18}O_8$	385.0989	385.0918	297–341	++
14.2	Epicatechin 3-O-galato	$C_{22}H_{18}O_{10}$	441.0984	441.0816	299	+++
15.2	4′-hydroxyflavanone	$C_{15}H_{12}O_3$	239.0736	239.0714	119	+
19.6	Sesamolinol (Isomer 1)	$C_{20}H_{20}O_7$	371.1578	371.1125	325–197	+
19.9	Syringic acid	$C_9H_{10}O_5$	197.1243	197.0444	na*	++
20.2	Sesamolinol (Isomer 2)	$C_{20}H_{20}O_7$	371.1572	371.1125	na	++
22.1	Ferulic acid	$C_{10}H_{10}O_4$	193.0301	193.0495	178	+
22.3	Sesamin	$C_{20}H_{18}O_6$	353.1227	353.1020	335–207	++
23.3	Episesamin	$C_{20}H_{18}O_6$	353.1223	353.1020	335–207	++
24.0	Diasesamin	$C_{20}H_{18}O_6$	353.1207	353.1020	335–223	+++
26.2	Genistein	$C_{15}H_{10}O_5$	269.0382	269.0444	195–223	+
29.6	Procyanidin dimer-type B	$C_{30}H_{26}O_{12}$	577.1378	577.1351	369	++
31.1	Sesamolin (Isomer 1)	$C_{20}H_{18}O_7$	369.0989	369.0969	339–277	++
34.2	Sesamolin (Isomer 2)	$C_{20}H_{18}O_7$	369.0987	369.0969	340	++
34.7	Sesamolin (Isomer 3)	$C_{20}H_{18}O_7$	369.0997	369.0969	340	++

Relative abundance (in base to % precursor ion intensity and % area) of the metabolites identified is indicated by + (0–5%), ++ (5–20%), +++ (20–50%) and ++++ (50–100%)

*Not available

Table 3 HPLC–ESI–MS/MS analysis of subcritical fluid extracts obtained from pistachio nuts cake

Rt (min)	Compound	Molecular formula	[M−H]⁻ experimental (m/z)	[M−H]⁻ theoretical (m/z)	[MS² M−H⁻] (m/z)	Relative abundance
12.8	Daidzein	$C_{15}H_{20}O_4$	253.0855	253.0799	na*	+
13.5	Gallic acid derivate (Isomer 2)	$C_{15}H_{26}O_{19}$	509.1017	509.0985	267	+++
14.0	Gallic acid derivate (Isomer 3)	$C_{17}H_{24}O_{11}$	403.1333	403.1235	313	++
16.9	Digalloyl hexose (Isomer 1)	$C_{20}H_{20}O_{14}$	483.0509	483.0789	331	+
17.3	Digalloyl hexose (Isomer 2)	$C_{20}H_{20}O_{14}$	483.0514	483.0789	331	++
19.9	Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0903	353.0867	191	++
20.5	Genistein -7-O-glucoside	$C_{21}H_{20}O_{10}$	431.1947	431.1973	269	+
22.4	Quercetin galloyl hexoside (Isomer 1)	$C_{28}H_{24}O_{16}$	615.1078	615.0981	301	++++
22.9	Quercetin-3-O-glucoside (Isoquercetin)	$C_{21}H_{20}O_{12}$	463.0816	463.0871	301	+
23.1	Quercetin galloyl hexoside (Isomer 2)	$C_{28}H_{24}O_{16}$	615.1099	615.0981	301	+++
23.8	Kaempferol hexoside (Isomer 1)	$C_{21}H_{20}O_{11}$	447.0916	447.0922	285	+
24.1	Kaempferol hexoside (Isomer 2)	$C_{21}H_{20}O_{11}$	447.0899	447.0922	285	+
26.3	Naringenin	C15H12O5	271.0656	271.0612	151	+++
27.9	Procyanidin dimer-type B (Isomer 1)	$C_{30}H_{26}O_{12}$	577.1302	577.1341	na	+
31.0	Procyanidin dimer-type B (Isomer 2)	$C_{30}H_{26}O_{12}$	577.1302	577.1341	na	++
33.2	(15:3)-Anacardic acid	$C_{22}H_{30}O_3$	341.2055	341.2111	297	++

Relative abundance (in base to % precursor ion intensity and % area) of the metabolites identified is indicated by + (0–5%), ++ 5–20%), +++ (20–50%) and ++++ (50–100%)

*Not available

Fig. 1 Effects of the dry extracts on *F. verticillioides* (M3125) growth rates on PDA agar at 25 °C for 7 days. Bars with different letters are statistically different (p < 0.05). **a** Peanut skin, **b** Sesame seed cake, **c** Pistachio nuts cake
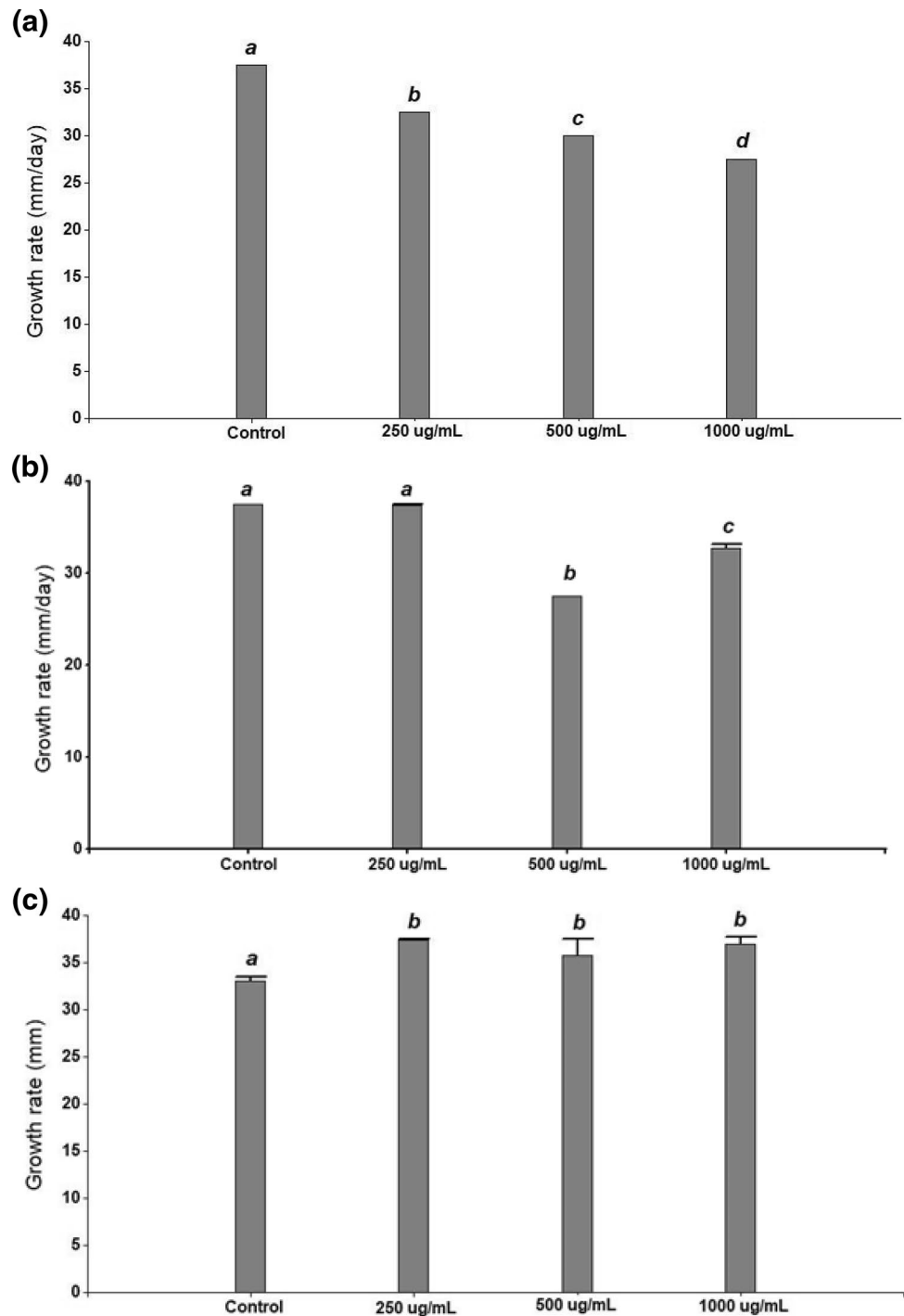
and dimeric (procyanidins dimers) flavonoids (Table 1). This agree partially with PS extracts obtained by means of conventional organic solvents under normal temperature and pressure conditions which show increased levels of higher molecular weight oligomers [24, 37].

Some previous studies highlight antimicrobial properties of low molecular weight flavonoid-type compounds. Shan et al. [41] reported extensive cell damage in pathogenic bacteria by using pure proanthocyanidins. Sarnoski

et al. [27] found that a fraction isolated from PS—containing mostly A-type procyanidin dimers—caused the highest growth inhibition percentage in yeast as compared to other fraction of higher molecular weight. These latter authors suggest that procyanidin dimers possess the ability to permeate through yeast cell wall due to their relatively low molecular weight (approximately 600 Da). As a result, these polyphenols could modify plasma membrane

structure, physical characteristics and functions (fluidity, metabolite fluxes) and affect cell division and growth.

The growth rates effect of the different SSC extract concentrations did not show a dose-dependent response (Fig. 1b). Nevertheless, a significant decrease in fungal growth rate was found at both the middle and the highest concentration levels (500 and 1000 μg/mL). These concentrations caused inhibition percentages near to 63% and 30%, respectively, in relation to the control treatment (data no shown). Some previous studies indicate that lignans may be related to resistance mechanisms against *Fusarium* spp. Esmaeilzadeh et al. [20] have reported that contamination with *F. graminearum* increases the production of lignans from *Linum album*. Hwang et al. [42] showed that pinoresinol (a lignan compound having phenolic aromatic rings) inhibits the growth of *Candida albicans* by causing damage to the fungal plasma membrane. Other studies revealed growth inhibitory effects of lignans against different fungi, including mycotoxin producers such as *Aspergillus flavus*, *A. niger* and *F. verticillioides* [22, 43]. The SSC extract used in the present study was composed mainly by lignans, most of them lacking phenolic structures. Although a clear relationship between structure and antifungal activity of lignan compounds has not been determined yet, some studies highlight growth inhibition effects of lignans bearing phenolic groups. So, it is possible that this latter condition (the presence of phenolic aromatic rings) may be associated to reductions in fungal growth rates as reported elsewhere [21–23].

The application of PNC extract did not have significant effect on *F. verticillioides* growth rate (Fig. 1c). Although we do not have an explanation at the moment, some studies [16, 35], suggest the lipophilicity as one of the most important chemical properties related to antifungal activity against *F. verticillioides*. Looking at the chemical compounds identified in PNC extracts (Table 1), it can be seen that many of them are glycosylated. This fact implies higher number of OH groups and, consequently, more polar and hydrophilic forms (i.e. lower lipophilicity) in relation to those found in PS extract.

The effect of the different extracts on the lag phase of *F. verticillioides* is showed in Fig. 2. All extracts prolonged this process and their effects were related directly with the concentrations tested. Other studies using PS extracts [26, 27] have shown significant increments of the lag phase in different microorganisms.

FB1 and Ergosterol Content in Maize Kernels

The effect of PS, SSC and PNC extracts on the FB_1 production is presented in Fig. 3a. Only SSC extract was found to be effective as antifumonisin agent. As compared to the control treatment, it reduced the mycotoxin production approximately 45%, 40% and 37% when used at 250, 500

and 1000 μg/mL, respectively. By using lignan-treated fungal cultures, Kulik et al. [21] have reported reductions in the synthesis of trichothecene—a mycotoxin produced by *F. graminearum*—which could be associated with decreased transcript levels for specific genes that are involved in the mycotoxin biosynthesis pathway.

Contrary to the results obtained with SSC, the extracts from PS and PNC caused stimulatory effects on mycotoxin production and increased significantly the FB_1 content in relation to the control treatment (Fig. 3a). This response has been also observed in other trials using artificially infected maize kernels in which fumonisins levels increased when phenolic and volatile compounds were used at high concentrations [26]. Such a response has been interpreted as a fungal survival mechanism [44] in which mycotoxin production is stimulated under certain stress conditions.

Some antifungal substances can inhibit fungal growth by interrupting the biosynthesis of ergosterol which is a key compound of the cell membrane structure in fungi [35]. All extracts evaluated here caused significant reductions in ergosterol content in relation to the control treatment (Fig. 3b). The lowest ergosterol contents were found by using SSC extracts; considering a mean value from the three concentrations tested, an average decrease of about 66% was found as compared to the control treatment. Similarly, PS and PNC extracts were found to decrease ergosterol content approximately 33% and 25% respectively, as compared to the control treatment.

Some chemical antifungal agents have been found to inhibit both the ergosterol and the fumonisin production. In the present study the lowest amount of ergosterol produced by *F. verticillioides* exposed to SSC extract coincided with the lower FB_1 production. As compared with PS and PNC extract components (mostly flavonoids and gallic acid derivates), the compounds present in SSC extract (mostly lignans) have more lipophilic character. It is known that lipophilicity may favor antifungals binding to ergosterol on the cellular membrane. This fact, in turn, may affect membrane integrity and function, and alter cell homeostasis [45]. The mechanism by which cell functions disturbances may cause mycotoxin depletion is yet unknown.

Conclusions

Agro-industrial by-products from peanut (PS), sesame (SS) and pistachio (PN) contain a vast array of bioactive compounds. Green solvent extraction using sub-critical fluids provides an efficient method to obtain extracts rich in phenolic and lignans compounds with potential use as antifungal agents. The effects of PS, SS and PN extracts on *F. verticillioides* growth rate and biochemical markers (fumonisin FB_1 and ergosterol production) were not univocal, and could be

Fig. 2 Effects of the dry extracts on the lag phase growth of *F. verticillioides* (M3125) on PDA agar at 25 °C for 7 days. Bars with different letters are statistically different ($p < 0.05$). **a** Peanut skin, **b** Sesame seed cake, **c** Pistachio nuts cake
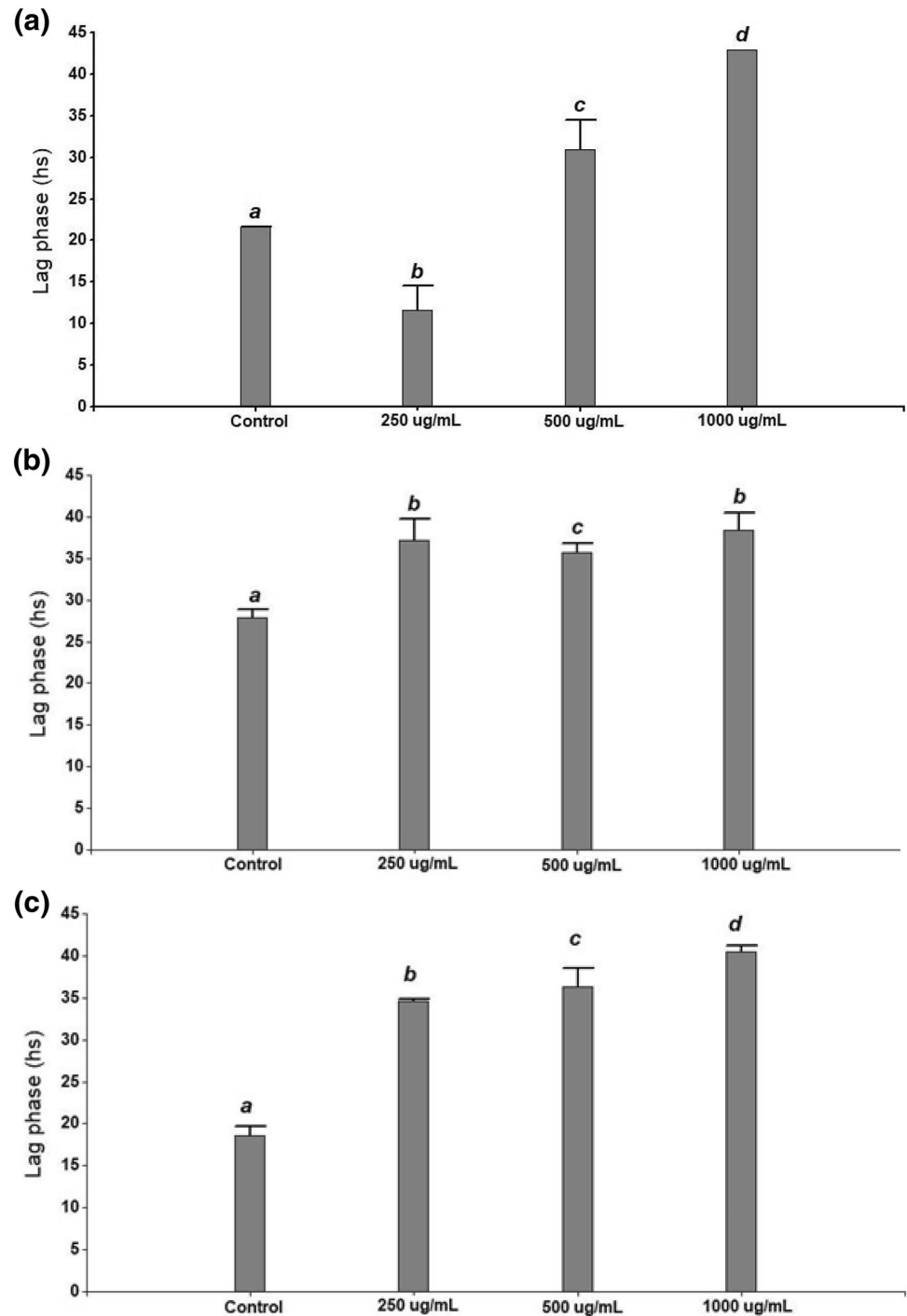

associated to differences in their chemical profiles. Extracts obtained from peanut skin—composed mainly by monomeric and dimeric flavonoids—caused significant reductions in fungal growth rate but increased FB_1 production. Extracts from sesame seeds—dominated by furofuran-type lignans—did not have a clear inhibitory effect on fungal growth rate but strongly reduced both FB_1 and ergosterol production. Extracts from pistachio nuts—characterized by monomeric

flavonoids and gallic acid derivatives—showed minor effects on both fungal growth rate and biochemical markers.

It is important to note that 'zero waste' objective can be reached by reusing the high value compounds from byproducts in innovative ways, which may generate profits in a future sustainable production system. In this sustainable and green context, studies like this and implementation of both, non-polluting extraction technologies and biopesticides

Fig. 3 Effects of the dry extracts on **a** FB$_1$ and **b** ergosterol content, in maize kernels after 28 days of incubation. Bars with different letters are statistically different (p < 0.05)
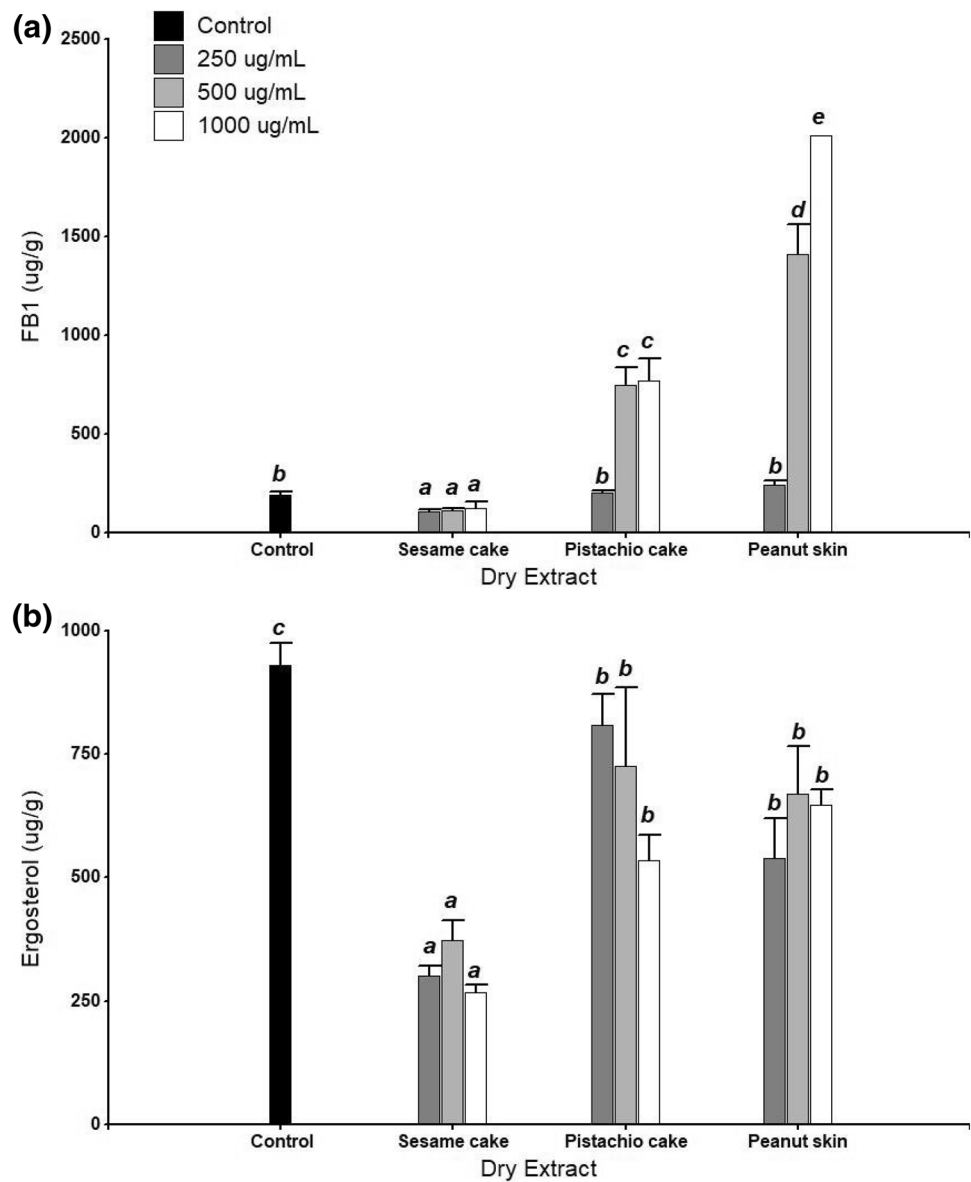
from natural source, add more value to the biocompounds recovery.

References

1. Banerjee, J., Singh, R., Vijayaraghavan, R., MacFarlane, D., Patti, A.F., Arora, A.: Bioactives from fruit processing wastes: green approaches to valuable chemicals. Food Chem. **225**, 10–22 (2017)

2. Lai, W.T., Khong, N.M.H., Lim, S.S., Hee, Y.Y., Sim, B.Y., Lau, K.Y., Lai, O.M.: A review: modified agricultural by-products for the development and fortification of food products and nutraceuticals. Trends Food Sci. Technol. **59**, 148–160 (2016)

3. Nazzaro, F., Fratianni, F., Ombra, M.N., D'Acierno, A., Coppola, R.: Recovery of biomolecules of high benefit from food waste. Curr. Opin. Food Sci. **22**, 43–54 (2018)

4. Sunil, L., Appaiah, P., Prasanth Kumar, P.K., Gopala Krishna, A.G.: Preparation of food supplements from oilseed cakes. J. Food Sci. Technol. **52**, 2998–3005 (2014)

5. Sarkis, J.R., Correa, A.P.F., Michel, I., Brandeli, A., Tessaro, I.C., Marczak, L.D.F.: Evaluation of the phenolic content and antioxidant activity of different seed and nut cakes from the edible oil industry. J. Am. Oil Chem. Soc. **91**, 1773–1782 (2014)

6. Terpinc, P., Ceh, B., Ulrih, N.P., Abramovič, H.: Studies of the correlation between antioxidant properties and the total

phenolic content of different oil cake extracts. Ind. Crops Prod. **39**, 210–217 (2012)

7. Ramachandran, S., Singh, S.K., Larroche, C., Soccol, C.R., Pandey, A.: Oil cakes and their biotechnological applications—a review. Bioresour. Technol. **98**, 2000–2009 (2007)

8. Meriles, J.M., Giorda, L.M., Maestri, D.M.: Effect of planting date on *Fusarium* spp. and *Diaporthe/Phomopsis* complex incidence and its relationship with soybean seed quality. J. Phytopathol. **150**, 606–610 (2002)

9. IARC: International Agency for Research on Cancer.: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, vol. 82, pp. 301–366. International Agency for Research on Cancer, Lyon (2002)

10. Agrios, G.: Plant Pathol, 5th edn. Elsevier Academic Press, Nueva York (2005)

11. Błaszczak-Świątkiewicz, K., Sikora, J., Szymański, J., Danilewicz, M., Mikiciuk-Olasik, E.: Biological evaluation of the toxicity and the cell cycle interruption by some benzimidazole derivatives. Tumor Biol. **37**, 11135–11145 (2016)

12. Seiber, J.N., Coats, J., Duke, S.O., Gross, A.D.: Biopesticides: state of the art and future opportunities. J. Agric. Food Chem. **62**, 11613–11619 (2014)

13. Barral, B., Chillet, M., Minier, J., Lechaudel, M., Schorrgalindo, S.: Evaluating the response to *Fusarium ananatum* inoculation and antifungal activity of phenolic acids in pineapple. Fungal Biol. **121**, 1045–1053 (2017)

14. Wang, S., Zheng, Y., Xiang, F., Li, S., Yang, G.: Antifungal activity of *Momordica charantia* seed extracts toward the pathogenic fungus *Fusarium solani* L. J. Food Drug Anal. **24**, 881–887 (2016)

15. Brado Avanço, G., Dias Ferreira, F., Silva Bomfim, N., De Souza, A., Rodrigues dos Santos, P., Peralta, R.M., Brugnari, T., Mallmann, C.A., de Abreu, Alves, Filho, B., Graton Mikcha, J.M., Machinski, M.: *Curcuma longa* L. essential oil composition, antioxidant effect, and effect on *Fusarium verticillioides* and fumonisin production. Food Control **73**, 806–813 (2017)

16. Dambolena, J.S., López, A.G., Meriles, J.M., Rubinstein, H.R., Zygadlo, J.A.: Inhibitory effect of 10 natural phenolic compounds on *Fusarium verticillioides*. A structure property activity relationship study. Food Control **28**, 63–70 (2012)

17. Da Silva, Bomfim N., Polis Nakassugi, L., Faggion Pinheiro Oliveira, J., Yumie Kohiyama, C., Aparecida, S., Mossini, G., Grespan, R., Botião Nerilo, S., Mallmann, C.A., Abreu Filho, B.A., Machinski, M.: Antifungal activity and inhibition of fumonisin production by *Rosmarinus officinalis* L. essential oil in *Fusarium verticillioides* (Sacc.) Nirenberg. Food Chem. **166**, 330–336 (2015)

18. Bodoira, R., Velez, A., Andreatta, A.E., Martinez, M., Maestri, D.: Extraction of bioactive compounds from sesame (*Sesamum indicum* L.) defatted seeds using water and ethanol under sub-critical conditions. Food Chem. **237**, 114–120 (2017)

19. Ben Othman, S., Katsuno, N., Kanamaru, Y., Yabe, T.: Water-soluble extracts from defatted sesame seed flour show antioxidant activity in vitro. Food Chem. **175**, 306–314 (2015)

20. Esmaeilzadeh, B.S., Sharifi, M., Behmanesh, M., Safaie, N., Murata, J., Araki, R., Yamagaki, T., Satake, H.: Time-course changes in fungal elicitor-induced lignan synthesis and expression of the relevant genes in cell cultures of *Linum album*. J. Plant Physiol. **169**, 487–491 (2012)

21. Kulik, T., Busko, M., Pszczółkowska, A., Perkowsk, J., Okorski, A.: Plant lignans inhibit growth and trichothecene biosynthesis in *Fusarium graminearum* Lett. Appl. Microbiol. **59**(99), 107 (2014)

22. Barbary, O.M., El-Sohaimy, S.A., El-Saadani, M.A., Zeitoun, A.M.A.: Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. Res. J. Agric. Biol. Sci. **6**, 247–256 (2010)

23. Nishiwaki, H., Nakazaki, S., Akiyama, K., Yamauchi, S.: Structure-antifungal activity relationship of fluorinated dihydroguaiaretic acid derivatives and preventive activity against *Alternaria alternata* Japanese pear pathotype. J. Agric. Food Chem. **65**, 6701–6707 (2017)

24. Ma, Y.Y., Cagnazzo, A.K., Kerr, W.L., Amarowicz, R., Swanson, R.B., Pegg, R.B.: Separation and characterization of phenolic compounds from dry-blanched peanut skins by liquid chromatography–electrospray ionization mass spectrometry. J. Chromatogr. A **1356**, 64–81 (2014)

25. Bodoira, R., Rossi, Y., Montenegro, M., Maestri, D., Velez, A.: Extraction of antioxidant polyphenolic compounds from peanut skin using water-ethanol at high pressure and temperature conditions. J. Supercrit. Fluid **128**, 57–65 (2017)

26. Pizzolitto, R.P., Dambolena, J.S., Zunino, M.P., Larrauri, M., Grosso, N.R., Nepote, V., Dalcero, A.M., Zygadlo, J.A.: Activity of natural compounds from peanut skins on *Fusarium verticillioides* growth and fumonisin B1 production. Ind. Crops Prod. **47**, 286–290 (2013)

27. Sarnoski, P., Boyer, R., O'Keefe, S.F.: Application of proanthocyanidins from peanut skins as a natural yeast inhibitory agent. J. Food Sci. **77**, 242–249 (2012)

28. Rajaei, A., Barzegar, M., Mobarez, A.M., Sahari, M.A., Esfahani, Z.H.: Antioxidant, anti-microbial and antimutagenicity activities of pistachio (*Pistachio vera)* green hull extract. Food Chem. Toxicol. **48**, 107–112 (2010)

29. Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.S., Abert-Vian, M.: Review of green food processing techniques. Preservation, transformation and extraction. Innov Food Sci. Emerg. Technol. **41**, 357–377 (2017)

30. Martínez, M.M., Bordón, M.G., Lallana, R.L., Ribotta, P.D., Maestri, D.M.: Optimization of sesame oil extraction by screw-pressing at low temperature. Food Bioprocess Tech. **10**, 1113–1121 (2017)

31. Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. **299**, 152–178 (1999)

32. Ersan, S., Ustundag, O.G., Carle, R., Schweiggert, R.F.: Identification of phenolic compounds in red and green pistachio (*Pistacia vera* L.) hulls (exo- and mesocarp) by HPLC-DAD-ESI-(HR)-MS. J. Agric. Food Chem. **64**, 5334–5344 (2016)

33. Leslie, J.F., Plattner, R.D., Desjardins, A.E., Klittich, C.J.: Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section Liseola). Phytopathology **82**, 341–345 (1992)

34. Shephard, G.S., Sydenham, E.W., Thiel, P.G., Gelderblom, W.C.A.: Quantitative determination of fumonisin B1 and B2 by high-performance liquid chromatography with fluorescence detection. J. Liq. Chromatogr. **13**, 2077–2087 (1990)

35. Dambolena, J.S., López, A.G., Canepa, M.C., Theumer, M.G., Zygadlo, J.A., Rubinstein, H.R.: Inhibitory effect of cyclic terpenes (limonene, menthol, menthone and thymol) on *Fusarium verticillioides* MRC 826 growth and fumonisin B1 biosynthesis. Toxicon **51**, 37–44 (2008)

36. Sichetti Munekata, P.E., Paseto Fernandes, R.P., Pires de Melo, M., Trindade, M.A., Lorenzo, J.M.: Influence of peanut skin extract on shelf-life of sheep patties. Asian Pac. J. Trop. Med. **6**, 586–596 (2016)

37. Sarnoski, P.J., Johnson, J.V., Reed, K.A., Tanko, J.M., O'Keefe, S.F.: Separation and characterisation of proanthocyanidins in Virginia type peanut skins by LC–MS. Food Chem. **131**, 927–939 (2012)

38. Moazzami, A.A., Andersson, R.E., Kamal-Eldin, A.: HPLC analysis of sesaminol glucosides in sesame seeds. J. Agric. Food Chem. **54**, 633–638 (2007)

39. Dar, A.A., Arumugam, N.: Lignans of sesame: Purification methods, biological activities and biosynthesis—a review. Bioorg. Chem. **50**, 1–10 (2013)

40. Nadeem, M., Situ, C., Mahmud, A., Khalique, A., Imran, M., Rahman, F., Khan, S.: Antioxidant activity of sesame (*Sesamum indicum* L.) cake extract for the stabilization of olein based butter. J. Am. Oil Chem. Soc. **91**, 967–977 (2014)

41. Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H.: Antibacterial properties of *Polygonum cuspidatum* roots and their major bioactive constituents. Food Chem. **109**, 530–537 (2008)

42. Hwang, B., Lee, J., Liu, Q., Woo, E., Lee, D.G.: Antifungal effect of (+)-pinoresinol isolated from *Sambucus williamsii*. Molecules **15**, 3507–3516 (2010)

43. Céspedes, C.L., Guillermo Avila, J., García, A.M., Becerra, J., Flores, C., Aqueveque, P., Bittner, M., Hoeneisen, M., Martinez, M., Silva, M.: Antifungal and antibacterial activities of *Araucaria araucana* (Mol.) K. Koch heartwood lignans. Zeitschrift für Naturforschung C **61**, 35–43 (2006)

44. Reynoso, M., Torres, A., Ramírez, M.L., Rodríguez, M., Chulze, S., Magan, N.: Efficacy of antioxidant mixtures on growth, fumonisins production and hydrolyticenzyme production by *Fusarium verticillioides* and *F. proliferatum* in vitro on maize-based media. Mycol. Res. **106**, 1093–1099 (2002)

45. Bendaha, H., Yu, L., Touzani, R., Souane, R., Giaever, G., Nislowc, C., Boone, C., El Kadiri, S., Brownb, G.W., Bellaoui, M.: New azole antifungal agents with novel modes of action: synthesis and biological studies of new tridentate ligands based on pyrazole and triazole. Eur. J. Med. Chem. **46**, 4117–4124 (2011)

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